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Sulphide oxidation to elemental sulphur in a membrane bioreactor: Performance and characterization of the selected microbial sulphur-oxidizing community☆

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Abstract

In leather tanning industrial areas sulphide management represents a major problem. However, biological sulphide oxidation to sulphur represents a convenient solution to this problem. Elemental sulphur is easy to separate and the process is highly efficient in terms of energy consumption and effluent quality. As the oxidation process is performed by specialized bacteria, selection of an appropriate microbial community is fundamental for obtaining a good yield. Sulphur oxidizing bacteria (SOB) represent a wide-ranging and highly diversified group of microorganisms with the capability of oxidizing reduced sulphur compounds. Therefore, it is useful to select new microbes that are able to perform this process efficiently. For this purpose, an experimental membrane bioreactor for sulphide oxidation was set up, and the selected microbial community was characterized by constructing 16S rRNA gene libraries and subsequent screening of clones. Fluorescence *in situ* hybridization (FISH) was then used to assess the relative abundance of different bacterial groups. Sulphide oxidation to elemental sulphur proceeded in an efficient (up to 79% conversion) and stable way in the bioreactor. Both analysis of clone libraries and FISH experiments revealed that the dominant operational taxonomic unit (OTU) in the bioreactor was constituted by *Gammaproteobacteria* belonging to the *Halothiobacillaceae* family. FISH performed with the specifically designed probe tios_434 demonstrated that this OTU constituted $90.6 \pm 1.3\%$ of the bacterial community. Smaller fractions were represented by bacteria belonging to the classes *Betaproteobacteria*, *Alphaproteobacteria*, *Deltaproteobacteria*, *Clostridia*, *Mollicutes*, *Sphingobacteria*, *Bacteroidetes* and *Chlorobia*. Phylogenetic analysis revealed that clone sequences from the dominant OTU formed a stable clade (here called the TIOS44 cluster), within the *Halothiobacillaceae* family, with sequences from many organisms that

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have not yet been validly described. The data indicated that bacteria belonging to the TIOS44 cluster were responsible for the oxidation process.

Keywords: Membrane bioreactors (MBR); Sulphur; Sulphide; Sulphur-oxidizing bacteria; *Halothiobacillaceae*; tios_434; Tios44 cluster; *Halothiobacillus*; *Thiovirga*; "*Thiobacillus baregensis*"

Introduction

High concentrations of sulphide compounds are usually present in wastewaters coming from many industrial treatments, such as leather tanning or papermaking. Under anoxic conditions, these compounds can also be produced from sulphates by biological reduction. Sulphide ions in the liquid phase can cause several problems in conventional activated sludge plants. Some of these negative effects are inhibition of nitrification [1,7,10], growth of filamentous bacteria [28] and floc disruption [63], as well as lower efficiency in sludge dehydration [44]. For these reasons, sulphide ions have to be removed before the treatment process. Moreover, high sulphide concentrations are always associated with hydrogen sulphide emissions that are toxic and, therefore, they have to be appropriately treated.

In leather tanning industrial areas sulphide management represents a major problem. In fact, gas streams need to be treated not only in wastewater treatment plants, but also in each single manufacturing plant. The area of S. Croce (Pisa, Italy) is one of the biggest leather tanning industrial districts in Europe, comprising about 400 tanneries. Many of these plants are equipped with a hydrogen sulphide treatment system, generally consisting of wet scrubbers using basic solutions (pH 10.5–13). Effluent solutions from these towers are characterized by a chemical oxygen demand (COD) concentration that is almost entirely due to sulphide ions. In these solutions, sulphide represents the main electron donor, as the organic component is extremely poor. All these features make this substrate particularly suitable for a treatment based on biological sulphide oxidation.

Sulphide oxidation can be limited to elemental sulphur production, without proceeding completely to sulphate production. This result can be achieved by maintaining a low oxygen concentration ($<0.1\,\mathrm{mg\,l^{-1}}$) in the bioreactor, usually by controlling the aeration through continuous monitoring of the oxidation–reduction potential (ORP) [27]. Elemental sulphur is easier to separate [26,33] and the process requires lower oxygen consumption, resulting in lower levels of acidification. Furthermore, sulphide oxidation to elemental sulphur is convenient in terms of energy consumption and effluent quality. If the process is efficiently performed in a basic environment, effluent solutions can also be reused in the wet scrubbers.

Given appropriate conditions of dissolved oxygen, sulphide concentration and absence of organic biodegradable compounds, it is possible to select an obligate chemolithoautotrophic biomass able to perform the sulphide oxidation process to elemental sulphur. In these conditions, it has already been shown that elemental sulphur production can account for 70% of incoming sulphide [8,9].

Besides chemical conditions, the technical features of the reactor also play an important role in solid separation and biomass selection. Selection of an appropriate microbial community has key relevance for the efficiency of the sulphide oxidation process. Sulphur oxidizing bacteria (SOB) represent a wide-ranging group of microorganisms with the capability of oxidizing reduced sulphur compounds to sulphates, with or without formation of sulphur globules as a reaction intermediate. The currently described SOB represent a highly diverse group. From a phylogenetic and taxonomic point of view, SOB species comprise prokaryotes belonging to several different and highly divergent lineages, such as *Crenarchaeota* and, among *Bacteria*, *Proteobacteria* as well as *Chloroflexi*, *Chlorobia* and *Spirochaetes* [43]. Heterogeneity of SOB is also evident with respect to life styles, as the group not only includes organisms adapted to extreme natural environments, such as hydrothermal vents or hypersaline habitats [54,55] and to man-made environments [6,24,61], but also contains symbionts and their free-living relatives [43]. The metabolism of these prokaryotes can also vary greatly, as both photo- and chemotrophic bacteria are included. Metabolic pathways used for biological oxidation of sulphur compounds naturally affect the ability to convert sulphides to elemental sulphur efficiently. It has been shown that the yield of the sulphide oxidation process depends on the features of the selected microbial community [60]. For this reason, and considering the wide heterogeneity of SOB, it is useful to select new microbial organisms, which are able to perform the oxidation of sulphides to elemental sulphur efficiently at high pH and high sulphide concentrations.

Therefore, an experimental bioreactor for oxidation of sulphides was set up and its microbial community was characterized. It was housed at the Consorzio Cuoiodepur Spa wastewater treatment plant in S. Croce (Pisa, Italy), as part of a regional project called *Tecnologie Innovative per l'Ossidazione biologica dei Solfuri* (TIOS)